Ability of Hydrogen Peroxide and Timsen to Eliminate Artificially Inoculated *Salmonella* from Fertile Broiler Eggs

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Primary Audience: Researchers, Hatchery Managers, Flock Supervisors

SUMMARY

Varying levels (10^5 to 10^7/egg) of *Salmonella* Typhimurium were inoculated onto fertile hatching eggs by immersion. After this, the inoculated eggs were untreated (control), water treated, hydrogen peroxide (1.5%) treated, or Timsen treated (n-alkyl dimethyl benzyl ammonium chloride as a commercial bactericide-fungicide). Hydrogen peroxide was superior to Timsen as an egg treatment to eliminate artificially inoculated *Salmonella* from fertile eggs, but one-third of the treated eggs remained *Salmonella* positive. This study demonstrates how difficult it is to eliminate *Salmonella* that contaminate fertile hatching eggs. Until a more effective system or process is devised and commercially implemented, *Salmonella* and other organisms will continue to pass from one generation to the next through the fertile egg.

Key words: broiler egg, hydrogen peroxide, *Salmonella*, Timsen

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DESCRIPTION OF PROBLEM

Laboratory studies have demonstrated that *Salmonella* can rapidly penetrate the freshly laid fertile hatching egg [1]. These invading *Salmonella* usually do not cause extensive decomposition to the fertile egg and the chick ultimately hatches [2, 3]. This invasion can result in the establishment of *Salmonella* reservoirs in commercial broiler [4] and breeder [5] hatcheries and in broilers populations [6]. Very low levels of *Salmonella* can colonize the intestinal tract of young broiler chicks [7, 8, 9] and unhatched embryos [10].

Other studies [11, 12, 13] have demonstrated that the *Salmonella* serovars found on the final product (fully processed broiler carcases) predominately originate from hatcheries and breeder flocks. These *Salmonella* reservoirs will continue to exist until these microorganisms are eliminated from broiler breeder flocks or eliminated from the freshly laid fertile egg by an effective and rapidly applied chemical treatment [14, 15].

The objective of this study was to evaluate the effectiveness of hydrogen peroxide or Timsen (N-alkyl dimethyl benzyl ammonium chloride) [16] to eliminate *Salmonella* from fertile

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TABLE 1. Effectiveness of Timsen and H_2O_2 treatments to eliminate *Salmonella Typhimurium* inoculated onto fertile eggs by fecal smear

	Rep 1[A]	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Overall
Control	15/15[B]	15/15	15/15	15/15	12/15	15/15	87/90 (97%)[a]
H_2O	15/15	15/15	15/15	15/15	1/15	15/15	76/90 (84%)[b]
Timsen	15/15	15/15	15/15	15/15	3/15	14/15	77/90 (86%)[b]
H_2O_2	0/15	0/15	3/15	0/15	0/15	3/15	6/90 (7%)[c]

[a–c]Rows with different letters are significantly different as determined by chi-squared test for independence.
[A]Rep = replicate.
[B]Number of *Salmonella*-positive eggs after treatment/number of eggs tested.

hatching eggs. Hydrogen peroxide is being commercially used in the United States, and Timsen is being widely used in South America.

MATERIALS AND METHODS

Freshly laid fertile eggs used in this study were from commercial broiler breeders raised under experimental conditions. Approximately 100,000 cells of a nalidixic-acid-resistant strain of *Salmonella* Typhimurium were inoculated onto freshly laid fertile hatching eggs by immersion [17, 18] or by fecal smear [19]. For the immersion inoculations, there were 15 fertile eggs per treatment with two replications. After eggs were warmed to 37°C they were immersed for 1 min in a 20°C bacterial suspension (~100,000 cells/mL). For the fecal smear inoculations, there were 15 eggs per treatments and six replications. After autoclaved feces were inoculated with ~100,000 cells/g, 1 g of material was smeared onto the surface of the eggs using a sterile swab. After inoculation, the eggs were held at room temperature for about 1 h to allow the eggs to dry prior to treatment.

Treatments were applied to inoculated eggs with a spray bottle. Each egg was sprayed with 10 mL of one of the following solutions: control (untreated), water, hydrogen peroxide (1.5% solution), or Timsen (0.8 mg/L). Treated eggs were allowed to dry for 1 h prior to microbiological sampling.

The procedure for recovery of the marker *S.* Typhimurium consisted of breaking the eggshell, discarding the contents, then gently crushing by hand the shell and adhering membranes in 50 mL of buffered peptone in a sterile plastic bag [19]. The bag and contents were then incubated for 24 h at 37°C. After the pre-enrichment step, a sterile cotton swab was soaked in the broth and streaked onto BG Sulfa agar [20] with 200 ppm sodium salt hydrate nalidixic acid [21]. After incubation at 37°C for 24 to 48 h, characteristic *Salmonella* colony-forming units appearing on the plate were serologically confirmed to be our marker organism.

RESULTS AND DISCUSSION

Table 1 shows the data obtained when the eggs were inoculated with 10^5 *S.* Typhimurium per egg by the fecal smear method. With the H_2O_2 treatment, the inoculated *S.* Typhimurium was eliminated from 84 of the 90 eggs treated, whereas Timsen eliminated the organism from only 13 of the 90 eggs. Results were analyzed by the chi-squared test for independence ($P < 0.05$) [22]. H_2O_2 was significantly different from all other treatments. However, water and Timsen treatments were significantly different from control treatments but not from each other.

Table 2 shows the data when a more stringent method of inoculation was used (the immersion procedure). Once again, although control and H_2O_2 treatment results differed significantly, neither water nor Timsen treatments differed significantly from either of the other two treatments. The most effective treatment, H_2O_2, eliminated *S.* Typhimurium from only 30% of the inoculated eggs.

In this study, H_2O_2 was superior to Timsen as a treatment for elimination of *S.* Typhimurium from fecal-smear-inoculated fertile eggs but less effective in removing the organism from fertile eggs when an immersion inoculation was used. In fact, Timsen was only slightly more effective than the water treatment. However, in contrast to our findings, Timsen has been shown to inhibit bacteria on poultry carcasses associated with spoilage [23]. Also, other research [24] has dem-

TABLE 2. Effectiveness of Timsen and H_2O_2 treatments to eliminate *Salmonella Typhimurium* inoculated onto fertile eggs by immersion

Treatment	Rep 1[A]	Rep 2	Overall
Control (none)	15/15[B]	15/15	30/30 (100%)[a]
H_2O	14/15	14/15	28/30 (93%)[ab]
Timsen	13/15	13/15	26/30 (87%)[ab]
H_2O_2	9/15	12/15	21/30 (70%)[b]

[a,b]Rows with different letters are significantly different as determined by chi-squared test for independence.
[A]Rep = replicate.
[B]Number of *Salmonella*-positive eggs after treatment/number of eggs tested.

onstrated that Timsen is superior to other disinfectants in commercial hatcheries in reducing microbial populations in hatcher room air and on surfaces. In those studies Timsen performed better than the hatchery's normal disinfectant 60% of the time. Timsen performed the same as the hatchery's standard operating procedure disinfectant 23% of the time and worse 17% of the time. Other advantages of Timsen in the commercial hatchery include its safety for humans and its significantly reduced corrosiveness when compared to other popular hatchery disinfectants.

Hydrogen peroxide has been consistently shown to be an effective chemical for the disinfection of fertile hatching eggs [25, 26, 27] and does not adversely affect hatchability [28], livability, body weight, or feed conversion [29]. Even though H_2O_2 is an effective treatment, 70% of the eggs remained contaminated with *Salmonella* after treatment when an immersion method of inoculation was used. This study demonstrates how difficult it can be to eliminate *Salmonella* that have penetrated fertile hatching eggs. Until an effective treatment is devised and applied, these bacteria will continue to pass from one generation to the next in commercial poultry through fertile eggs.

CONCLUSIONS AND APPLICATIONS

1. Hydrogen peroxide was superior to Timsen for eliminating *Salmonella* Typhimurium from hatching eggs.
2. *Salmonella* Typhimurium are difficult to eliminate from fertile eggs when significant penetration of the shell and membranes occur.
3. Until an effective and rapidly applied chemical treatment is devised and widely used in commercial breeder and broiler hatcheries, bacteria will continue to pass from one generation to the next through fertile eggs.

REFERENCES AND NOTES

1. Williams, J. E., and L. H. Dillard. 1968. *Salmonella* penetration of fertile and infertile chicken eggs at progressive stages of inoculation. Avian Dis. 12:629–635.

2. MacLaury, D. W., and A. B. Moran. 1959. Bacterial contamination of hatching eggs. Kentucky Agric. Exp. Sta. Bull. 665. Lexington, KY.

3. Cason, J. A., N. A. Cox, and J. S. Bailey. 1991. Survival of *Salmonella typhimurium* during incubation and hatching of inoculated eggs. Poult. Sci. 70(Suppl. 1):152. (Abstr.)

4. Cox, N. A., J. S. Bailey, J. M. Mauldin, and L. C. Blankenship. 1990. Presence and impact of *Salmonella* contamination in commercial broiler hatcheries. Poult. Sci. 69:1606–1609.

5. Cox, N. A., J. S. Bailey, J. M. Mauldin, L. C. Blankenship, and J. L. Wilson. 1991. Extent of salmonellae contamination in breeder hatcheries. Poult. Sci. 70:416–418.

6. Blankenship, L. C., J. S. Bailey, N. A. Cox, N. J. Stern, R. Brewer, and O. Williams. 1993. Two-step mucosal competitive exclusion flora treatment to diminish *Salmonella* in commercial broiler chickens. Poult. Sci. 72:1667–1672.

7. Milner, K. C., and M. F. Shaffer. 1952. Bacteriologic studies of experimental *Salmonella* infections in chicks. J. Infect. Dis. 90:81–85.

8. Schleifer, J. J., B. J. Juven, C. W. Beard, and N. A. Cox. 1984. The susceptibility of chicks to *Salmonella montevideo* in artificially contaminated poultry feed. Avian Dis. 28:497–503.

9. Cox, N. A., J. S. Bailey, L. C. Blankenship, R. J. Meinersmann, N. J. Stern, and F. McHan. 1990. Colonization dose 50% values for *Salmonella* administered orally and intracloacally to young broiler chicks. Poult. Sci. 69:1809–1812.

10. Cason, J. A., J. S. Bailey, and N. A. Cox. 1992. Localization of *Salmonella typhimurium* during incubation and hatching of inoculated eggs. Poult. Sci. 71(Suppl. 1):143. (Abstr.)

11. Lahellec, C., and P. Colin. 1985. Relationship between serotype of salmonellae from hatcheries and rearing farms and those from processed poultry carcasses. Br. Poult. Sci. 26:179–186.

12. Goren, E., W. A. De Jong, P. Dorrenbal, N. M. Bolder, R. W. A. W. Mulder, and A. Jansen. 1988. Reduction of salmonellae infection of broilers by spray application of intestinal microflora: A longitudinal study. Vet. Q. 10:249–255.

13. Bailey, J. S., N. A. Cox, L. C. Blankenship, and N. J. Stern. 1991. Effect of competitive exclusion microflora on the distribution of *Salmonella* serotypes in an integrated poultry operation. Poult. Sci. 70(Suppl. 1):15. (Abstr.)

14. Cox, N. A., and J. S. Bailey. 1991. Efficacy of various chemical treatments to eliminate *Salmonella* on hatching eggs. Poult. Sci. 70(Suppl. 1):31. (Abstr.)

15. Cox, N. A., and J. S. Bailey. 1992. Chemical treatment of fertile hatching eggs to control *Salmonella* at the breeder flock and hatchery level. Poult. Sci. 71(Suppl. 1):7. (Abstr.)

16. Biomedical Development Corp., San Antonio, TX.

17. Cox, N. A., J. S. Bailey, and M. E. Berrang,1998. Bactericidal treatment of hatching eggs I. Chemical immersion treatments and *Salmonella*. J. Appl. Poult. Res. 7:347–350.

18. Cox, N. A., M. E. Berrang, R. J. Buhr, and J. S. Bailey. 1999. Bactericidal treatment of hatching eggs. II. Use of chemical disinfectants with vacuum to reduce *Salmonella*. J. Appl. Poult. Res. 8:321–326.

19. Berrang, M. E., N. A. Cox, J. S. Bailey, and L. C. Blankenship. 1991. Methods for inoculation and recovery of Salmonella from chicken eggs. Poult. Sci. 70:2267–2270.

20. Difco, Division of Becton-Dickinson, Sparks, MD.

21. Aldrich, St. Louis, MO.

22. Ott, L. 1977. Count Data. Pages 265–305 in An Introduction to Statistical Methods and Data Analysis. Duxbury Press, Belmont, CA.

23. Russell, S. M. 1998. Chemical sanitizing agents and spoilage bacteria on fresh broiler carcasses. J. Appl. Poult. Res. 7:273–280.

24. Mauldin, J. M. 1999. Poultry Science Extension, University of Georgia, Athens, GA. Personal communication.

25. Cox, N. A., J. S. Bailey, M. E. Berrang, R. J. Buhr, and J. M. Mauldin. 1994. Chemical treatment of Salmonella-contaminated fertile hatching eggs using an automated egg spray sanitizing machine. J. Appl. Poult. Res. 3:26–30.

26. Padron, M. 1995. Egg dipping in hydrogen peroxide solution to eliminate *Salmonella typhimurium* from eggshell membranes. Avian Dis. 39:627–630.

27. Shane, S. M., and A. Faust. 1996. Evaluation of sanitizers for hatching eggs. J. Appl. Poult. Res. 5:134–138.

28. Cox, N. A., M. E. Berrang, and R. J. Buhr. 1998. Hydrogen peroxide applied with vacuum and a surfactant to eliminate *Salmonella* from hatching eggs. Poult. Sci. 77(Suppl. 1):20. (Abstr.)

29. Sander J. E., and J. L. Wilson. 1999. Effect of hydrogen peroxide disinfection during incubation of chicken eggs on microbial levels and productivity. Avian Dis. 43:227–233.

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